TECHNICAL MANUSCRIPT 224

ETHYLENE PRODUCTION FROM LINOLENIC ACID

Frederick B. Abeles

MAY 1965

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 224

ETHYLENE PRODUCTION FROM LINOLENIC ACID

Frederick B. Abeles

Crops Division
DIRECTORATE OF BIOLOGICAL RESEARCH

Project 1A014501A91A

May 1965

ABSTRACT

The possibility of linolenic acid serving as the precursor of ethylene in vivo was investigated. A comparison of the amount of linolenic acid present in plant tissues with the rates of ethylene production indicated that linolenic acid does not serve as the source of ethylene in plants.

ETHYLENE PRODUCTION FROM LINOLENIC ACID

Lieberman and Mapson[1] have shown that linolenic acid may serve as a precursor of ethylene. The possibility that the ethylene that is evolved from plants arises from linolenic acid was investigated by determining the relationship between ethylene evolution and linolenic acid levels from a number of plants.

Ethylene production from linolenic acid was measured in a manner similar to one described earlier.[2] The fatty acid peroxides were measured according to the methods of Dahle et al.[3] Extraction and measurement of fatty acids from plants followed the method of Huston and Albro.[4] Ethylene evolution from intact plants was measured by placing material into three-liter containers and withdrawing samples of gas after 20 hours. Measurement of ethylene by gas chromatography has also been described earlier.[5] Etiolated beans (Phaseolus vulgaris L. var. red kidney) and peas (Pisum sativum L. var. alaska) were grown in the dark for a week at 25 C and 80% relative humidity. Apples (Malus pumila Mill. var. eastern delicious) were obtained from local markets.

Figure 1 presents the results of a representative experiment showing the production of ethylene from peroxidized methyl linolenate. Other hydrocarbon gases such as ethane and propane, and a number of unidentified peaks were also produced. The presence or absence of a methyl group on the fatty acid has no effect on ethylene production. Under the conditions shown in Figure 1, the amount of ethylene evolved after 24 hours represents a 0.2% yield on a mole of ethylene per mole of peroxidized emolinate basis. The effect of ascorbate as a catalyst could be duplicated by 0.1 mg of a crude pea protein fraction described earlier[2] or similar amounts of lipoxidase. In these cases the activity of the protein was destroyed by heating. Addition of 1 μmole of KCN and 0.25 μmole of ethylenediaminetetraacetic acid inhibited gas production. A pH optimum between 7 and 8 was obtained with phosphate buffer; other buffers, such as pyrophosphate, tris(hydroxymethyl)aminomethane, and glycylglycine lowered the rate of gas production.

The possibility that ethylene production from peroxidized linolenate was mediated by means of a free radical mechanism was tested by the addition of 10 μmoles of catechol or I_2. Both of these compounds are known to quench free radical reactions by preferentially trapping the unpaired electrons. Both catechol and I_2 inhibited ethylene production from pea protein and ascorbate catalyzed reactions. In the case of the pea protein reaction inhibition was about 70% and for the ascorbate reaction inhibition was about 85%.

Other fatty acids were examined for their ability to serve as a substrate for this reaction. Stearic, oleic, linoleic, and arachidonic were found to be inactive. However, a C 22 fatty acid from herring oil, clupanodonic acid, was found to be active. The similarity between this compound and linolenic acid is the presence of a terminal CH_3-CH_2-CH= group at the methyl carbon end of the chain. On the premise that this particular end group accounts for the activity of linolenic acid, other compounds having the same structure were examined. 2-Pentenoic acid, 3-hexenoic acid, and 2-heptene were also active as a source of ethylene. Under conditions identical to those in Figure 1, ethylene production from these compounds was in the order of one nl (nanoliter) ethylene after 30 minutes.

Levels of linolenic acid and rates of ethylene production from apples were determined to explore the suggestion of Lieberman and Mapson[1] that ethylene production from apples may arise from linolenic acid. Market apples were peeled, cored, and sliced into quarter sections. Two slices were stored in a freezer and the other two placed in a container and the ethylene evolution measured after 20 hours. A representative chromatogram of the fatty acids extracted from apples is shown in Figure 2. The data from two experiments in Table 1 indicate that the linolenic acid content of apples remains approximately constant during this 24-hour period. The gas production during this time is equivalent to about 20% of the linolenic acid content. If linolenic acid is the source of ethylene in this tissue then the reaction is far more efficient in apples than in vitro. The data also indicate that the amount of linolenate does not change greatly during the period of time ethylene production was measured and also that a C 16 fatty acid with two double bonds is not present in the fatty acids extracted from the apple. If ethylene does arise from linolenic acid it does so from a small pool of activated (peroxidized) acid whose level is maintained by a rapid turnover of the activated fatty acid.

Naphthaleneacetic acid (NAA) is known to stimulate ethylene production from peas and beans.[5] It was of interest then to determine if increased rates of ethylene production would have any effect on linolenic acid levels from plants treated with NAA. Week-old etiolated pea and bean (cotyledons removed) plants were sprayed with 2×10^{-3} M NAA until runoff. Treated plants and controls were placed in containers for 20 hours, after which time ethylene evolution was measured and fatty acids were extracted. Representative chromatograms of the fatty acids of these plants are shown in Figures 3 and 4. No significant change was noted in the levels of any particular fatty acid between treated and control plants. Although care was taken to have the extraction of fatty acids quantitative, it was not possible to tell if changes in ethylene production between treated and controls were reflected in levels of linolenic acid (Table 2).

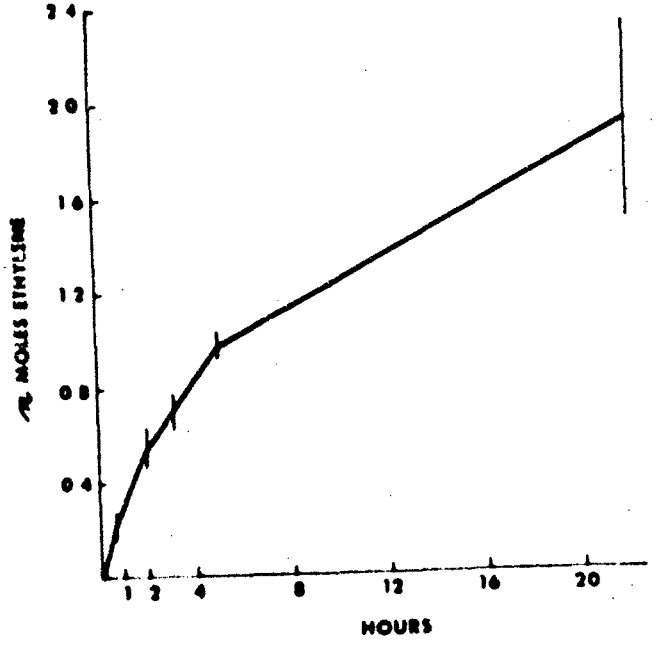

Figure 1. Ethylene Production from Peroxidized Linolenic Acid. 0.88 μmole peroxidized (0.06 mole O_2/mole fatty acid) linolenic acid; 50 μmoles sodium ascorbate; 125 μmoles potassium phosphate buffer, pH 7.3; liquid volume, 2.5 ml; helium gas phase, 25 C. Vertical lines indicate extent of standard error.

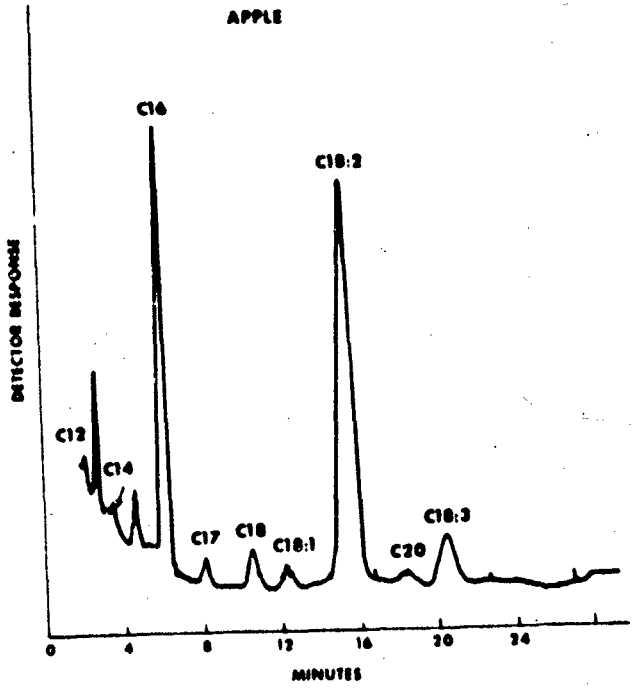

Figure 2. Fatty Acids from Malus pumila Mill.

TABLE 1. COMPARISON OF ETHYLENE PRODUCTION AND LINOLENIC ACID LEVELS FROM *MALUS PUMILA* MILL. VAR. EASTERN DELICIOUS

Experiment No.	Linolenic Acid, nmoles Fresh Weight, grams		Ethylene at 20 hrs, nmoles Fresh Weight, grams
	0 hours	24 hours	
1	50	33	8.8
2	32	48	15

TABLE 2. COMPARISON OF LINOLENIC ACID LEVELS AND ETHYLENE PRODUCTION FROM NAA-TREATED PLANTS

Experimental Material	Linolenic Acid, nmoles Fresh Weight, grams		Ethylene at 20 hrs, nmoles Fresh Weight, grams	
	Control	2×10^{-3} M NAA	Control	2×10^{-3} M NAA
Pisum sativum L.	340	130	0.05	7.1
Pisum sativum L.	140	260	0.05	2.2
Phaseolus vulgaris L.	570	480	0.18	14

The results with clupanodonic acid and other compounds containing terminal CH_3-CH_2-CH= groups indicated that the activity of linolenic acid is due only to this particular configuration. The possibility that ethylene may arise from this particular type of structure in nature remains open. Observing that a variety of other compounds such as ethylene oxide, diethyl ether, ethanol, and methionine have been reported[1] to produce ethylene suggests other alternatives. Any scheme that postulates linolenic acid as a precursor of ethylene in apples is unattractive because, first, the in vitro reaction is inefficient (0.02% after 20 hours) and the ratio of ethylene production to linolenic acid levels in apple is high (20% after 20 hours); and second, no C 16 fatty acid with two double bonds occurs in apple tissue. The fact that linolenic acid must be peroxidized[1] before it serves as a source of ethylene raises the question of whether fatty acid peroxides occur in normal tissues. Desai and Tappel[6] have shown that enzymes and subcellular particles are damaged by peroxidized fatty acids. However, there are numerous reports that peroxidized fats do occur in vivo.[7]

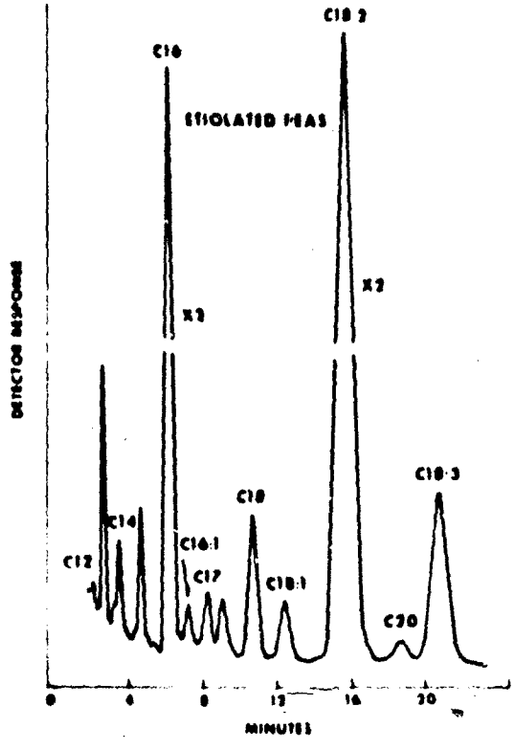

Figure 3. Fatty Acids from Etiolated Pisum sativum L.

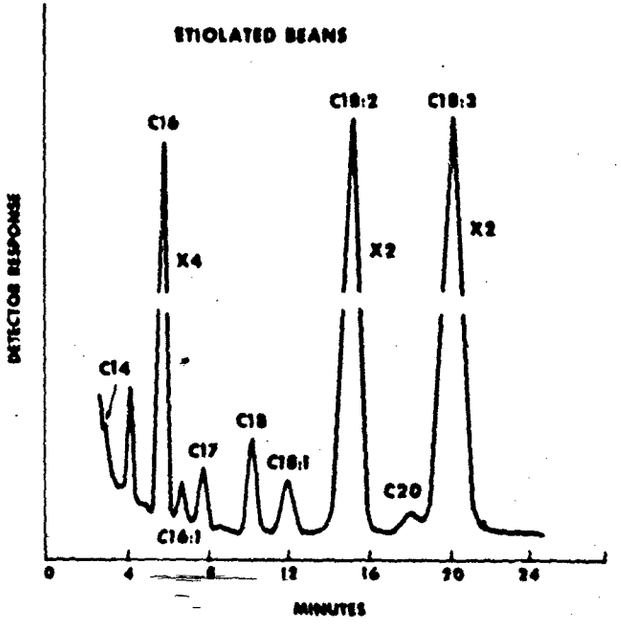

Figure 4. Fatty Acids from Etiolated Phaseolus vulgaris L.

In conclusion, the evidence indicates that linolenic acid does not serve as a precursor of ethylene. Final proof of this depends on measurements of rates of linolenic acid turnover and the extent to which peroxidized linolenic acid occurs in nature.

LITERATURE CITED

1. Lieberman, M., and L.W. Mapson. 1964. Genesis and biogenesis of ethylene. Nature 204:343-345.

2. Abeles, F.B., and B. Rubinstein. 1964. Cell-free ethylene evolution from etiolated pea seedlings. Biochem. Biophys. Acta. 93:675-677.

3. Dahle, L.K., E.G. Hill, and R.T. Holman. 1962. The thiobarbituric acid reaction and the autoxidation of polyunsaturated fatty acid methyl esters. Arch. Biochem. Biophys. 98:253-261.

4. Huston, C.K., and P.W. Albro. 1964. Lipids of Sarcina lutea: I. Fatty acid composition of the extractable lipids. J. Bacteriol. 88:425-432.

5. Abeles, F.B., and B. Rubinstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39:963-969.

6. Desai, F.D., and A.L. Tappel. 1963. Damage of proteins by peroxidized lipids. J. Lipid Res. 4:204-207.

7. Mead, J.F. 1961. Autoxidation induced by ionizing radiation, p. 299 to 323. In W.O. Lundberg (ed.) Autoxidation and antioxidants, Vol. I. Interscience Publications, New York.

DISTRIBUTION LIST

ADDRESSEE	NUMBER OF COPIES
Assistant Scientific Director Building 812	1
Directorate of Biological Research Building 560	1
Directorate of Industrial Health & Safety Building 550	1
Chief, Program Coordination Office Building 812	1
Chief, Medical Bacteriology Division Building 560	1
Chief, Medical Investigation Division Building 604	1
Chief, Physical Sciences Division Building 568	1
Chief, Process Development Division Building 469	1
Chief, Technical Evaluation Division Building 568	1
Chief, Virus & Rickettsia Division Building 539	1
Chief, Biomathematics Division Building 1422	1
Documents, Technical Library Building 426	1
Chief, Test Sphere Branch Technical Evaluation Division Building 568	1
Test Chamber Branch Technical Evaluation Division Building 1412	1
Technical Releases Branch Technical Information Division Building 426	10

ADDRESSEE	NUMBER OF COPIES
Chief, Crops Division Building 1301	10
Editorial Branch Technical Information Division Building 816	1
Assistant Director of Biological Engineering Building 722	1
Liaison Representative/Animal Disease Investigations Building 1301	1
U.S. Public Health Service Liaison Office Building 1301	1
Commanding Officer U.S. Naval Unit Building 125	1
Commander U.S. Army Edgewood Arsenal ATTN: SMUEA-CS Edgewood Arsenal, Maryland, 21010	1
Commander U.S. Army Chemical Research & Development Laboratories ATTN: Librarian Edgewood Arsenal, Maryland, 21010	2
Commanding General U.S. Army Munitions Command ATTN: AMSMU-SS-CS Dover, New Jersey, 07801	1
Commanding General U.S. Army Munitions Command ATTN: AMSMU-RE-R Dover, New Jersey, 07801	1
Commanding General U.S. Army Materiel Command Research Division, AMCRD-RC R&D Directorate Washington, D.C., 20315	1
Defense Documentation Center Cameron Station Alexandria, Virginia, 22314	20

ADDRESSEE	NUMBER OF COPIES
Detachment 4, RTD (ATCB) Eglin Air Force Base, Florida, 32542	1
APGC (PGBAP-1) Eglin Air Force Base, Florida, 32542	1
Dr. S.H. Madin Scientific Director Naval Biological Laboratory Naval Supply Center Oakland 14, California, 94614	1
Commanding Officer and Director U.S. Naval Applied Science Laboratory Naval Base, Code 9440 Brooklyn, New York, 11251	1
U.S. Army Standardization Group-Canada Office, Senior Standardization Rep. c/o Director of Equipment Policy Canadian Army Headquarters Ottawa 4, Canada	1
Munitions/TW Defence Research Staff British Embassy 3100 Massachusetts Avenue, N.W. Washington 8, D.C.	3
Canadian Liaison Office (CBR) building 5101 Edgewood Arsenal, Maryland, 21010	3
Australian Embassy ATTN: Lt. Col. P.D. Yonge Australian Army Staff (W) 2001 Connecticut Avenue, N.W. Washington 7, D.C.	2

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author) U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland, 21701	2a REPORT SECURITY CLASSIFICATION Unclassified 2b GROUP

3 REPORT TITLE

ETHYLENE PRODUCTION FROM LINOLENIC ACID

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

5 AUTHOR(S) (Last name, first name, initial)

Abeles, Frederick B.

6 REPORT DATE	7a TOTAL NO OF PAGES	7b NO OF REFS
May 1965	15 pages	7

8a CONTRACT OR GRANT NO b PROJECT NO 1A014501A91A c d	9a ORIGINATOR'S REPORT NUMBER(S) Technical Manuscript 224 9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10 AVAILABILITY LIMITATION NOTICES

Qualified requestors may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland, 21701

13 ABSTRACT

The possibility of linolenic acid serving as the precursor of ethylene in vivo was investigated. A comparison of the amount of linolenic acid present in plant tissues with the rates of ethylene production indicated that linolenic acid does not serve as the source of ethylene in plants.

DD FORM 1473

Unclassified
Security Classification